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PNEUMOCOCCAL MENINGITIS IN A RHESUS MONKEY.(U)
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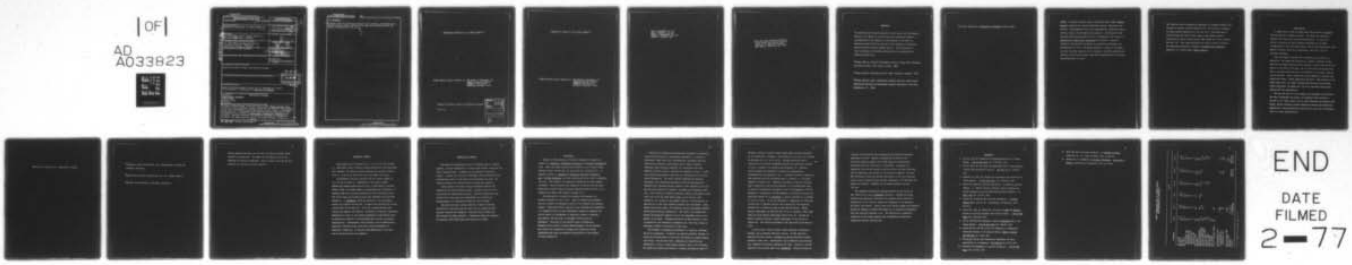

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A recently acquired young, 2.8-kg male rhesus monkey (Macaca mulatta) demonstrated a peculiar behavioral pattern, depression, and anorexia. Approximately 40 hr later, hyperesthesia, generalized muscle tremors, ataxia, and nystagmus became apparent. Cerebrospinal fluid contained numerous gram-positive cocci; subsequently, pure cultures of Streptococcus (Diplococcus) pneumoniae were isolated. Extensive antibiotic and supportive treatment for pneumococcal meningitis was administered for 12 da. The monkey appeared to respond to (cont'd)

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PNEUMOCOCCAL MENINGITIS IN A RHESUS MONKEY[1,2]

Please address reprint requests to: MAJ Donald G. Harrington, VC
Animal Assessment Division
USAMRIID, Fort Detrick
Frederick, Maryland 21701

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Please address reprint requests to: MAJ Donald G. Harrington, VC
Animal Assessment Division
USAMRIID, Fort Detrick
Frederick, Maryland 21701

ROY T. FAULKNER[3], BS, DVM
GARY T. BURGER[4], BS, DVM
DONALD G. HARRINGTON, DVM, MS
ROBERT L. HICKMAN,[5] DVM

[1]From the Animal Resources Division,
United States Army Medical Research
Institute of Infectious Diseases
Fort Detrick, Frederick, MD 21701

FOOTNOTES

[2]In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

[3]Present address: Clinical Investigation Service, Dwight David Eisenhower Army Medical Center, Fort Gordon, Georgia 30905.

[4]Present address: Pathology Services, NCTR, Jefferson, Arkansas 72079.

[5]Present address: Chief, Pathobiology Research Division, United States Army Medical Research and Development Command, Department of the Army, Washington, D.C. 20314.

Key words: Meningitis, Streptococcus pneumoniae, Rhesus monkey

SUMMARY A recently acquired young, 2.8-kg, male rhesus monkey (Macaca mulatta) demonstrated a peculiar behavioral pattern, depression, and anorexia. Approximately 40 hr later, hyperesthesia, generalized muscle tremors, ataxia, and nystagmus became apparent. Cerebrospinal fluid contained numerous gram-positive cocci; subsequently, pure cultures of Streptococcus (Diplococcus) pneumoniae were isolated. Extensive antibiotic and supportive treatment for pneumococcal meningitis was administered for 12 da. The monkey appeared to respond to treatment, but 9 da following cessation of all treatment, the monkey demonstrated residual central nervous system signs which progressed until the animal was euthanatized 3 wk later.

Most reported cases of pneumococcal meningitis in nonhuman primates have occurred in recently acquired animals (1-3). The success of treatment in simian primates apparently is very low (1,2). The monkey may be treated through the initial acute stage of the disease; however, incapacitating central nervous system (CNS) damage due to the infection may occur (1). This report describes the clinical course, treatment, and subsequent pathological findings of Streptococcus pneumoniae meningitis in a rhesus monkey (Macaca mulatta).

CASE HISTORY

A young, male, 2.8-kg, preconditioned rhesus monkey (M. mulatta) was received from a commercial vendor. The animal had undergone 3 negative tuberculin tests during preconditioning. One week after receipt, the animal was given ketamine hydrochloride (10 mg/kg; intramuscularly, IM), tuberculin-tested, treated with thiabendazole (100 mg/kg), tattooed, dusted for ectoparasites, and bled (7 ml) for serologic screening.

The first signs of illness (da 1) appeared on the 13th day of quarantine. The monkey was observed in a lateral recumbent posture. Each time the animal was disturbed or aroused it would stand upright and hold on to the side of the cage. Two to 3 min later, the monkey would drop to the cage bottom and lie on its sternum as if in pain. Anorexia was also evident. Pulse, respiration, mucous membranes, lung and heart sounds were normal. Rectal temperature was 103°F. When palpated in the right lumbar area, the animal displayed paravertebral muscle spasms. Sodium cephalothin (70 mg/kg) and 1 ml of an injectable multivitamin preparation[6] were administered.

The next day (da 2), a blood sample and radiographs were obtained. The chest radiographs were normal, but abdominal films revealed a dilated (2 to 3 times normal) loop of large intestine and retained fecal masses. Further abdominal studies demonstrated marked gastrointestinal hypomotility. Barium administered orally did not pass into the duodenum until 6 hr after administration.

and at 18 hr some barium was still present in the stomach. Sodium cephalothin and the multivitamin preparation were continued.

On da 3 the monkey was extremely hyperesthetic, ataxic, and uncoordinated. When handled, it displayed generalized muscle tremors and nystagmus. Cerebrospinal fluid (CSF) was collected from the cisterna magnum under ketamine hydrochloride sedation (10 mg/kg) and a second hematologic evaluation was performed. An indwelling catheter[7] was placed percutaneously into the left saphenous vein, and a dextrose-amino acid solution[8] mixed with equal parts of lactated Ringer's solution was infused by a peristalic pump[9] at 40 ml/hr. A 24-hr infusion of intravenous (IV) penicillin (2,000,000 units/da) was continuously maintained for 10 da. Chloromycetin (100 mg/kg), divided into 3 daily doses, was also given IV for 7 da. Potassium chloride (20 mEq/500 ml of infusate) was administered for the first 48 hr. Soduim pentobarbital was given IV, as needed, for sedation during the first 36 hr after neurological signs appeared. After 48 hr of treatment, the monkey had improved markedly, was transferred into a conventional primate restraint chair, and began to eat. Lactated Ringer's solution (15 ml/hr) was substituted as the infusate for the remaining 8 da of penicillin infusion. The animal continued to improve, the catheter was removed after 10 days of continuous IV penicillin, and the monkey was returned to its cage on day 13. IM penicillin (750,000 units/da) was then given for 2 additional days. A second cerebrospinal fluid tap (Table 1) was performed 9 da (da 23) after the final IM penicillin injection. At this time, the monkey had reduced ability to grasp with his left hand. This

[6]Betalin, Eli Lilly and Co., Indianapolis, Indiana

[7]Intrafusor, McGraw Laboratories, Inc., Milledgeville, Georgia and Glendale, California.

[8]Amino-Plex, Bio-ceutic Laboratories, Inc., St. Joseph, Missouri.

[9]IVAC-500, IVAC Corporation, San Diego, California.

deficit gradually worsened over the next 3 wk until the monkey became completely uncoordinated. The monkey was euthanatized (da 43) and submitted for necropsy examination. Prior to death, blood and CSF were collected for laboratory analysis (Table 1).

LABORATORY FINDINGS

Blood samples were collected on da 1, 3, 5, 16, 23 and 43 (Table 1). Significant initial findings included leukocytosis and hypokalemia. With treatment, the serum potassium concentration returned to normal on da 5. On da 16 the white blood cell count (WBC) was normal.

Cerebrospinal fluid was collected from the cisterna magnum on da 3, 23 and 43 (Table 1). Examination of the initial sample demonstrated numerous gram-positive cocci, a significantly increased number of WBC, an abnormal number of polymorphonuclear leukocytes, an unusually high total protein concentration, and a low glucose level. The low CSF sugar and elevated protein were consistent with bacterial meningitis. S. pneumoniae, which was sensitive to all antibiotics tested, was cultured from the CSF. No growth was obtained from a blood culture taken at the same time. On da 23, a second CSF smear and culture were negative for bacteria. Differential staining of leukocytes demonstrated a return to the normal predominance of mononuclear cells. An insufficient quantity of CSF prohibited WBC, protein, and glucose determinations. Cerebrospinal fluid obtained on da 43 demonstrated a moderately elevated protein level with a normal predominance of mononuclear leukocytes. No bacteria were demonstrated on the smear and the bacterial culture was negative.

PATHOLOGICAL FINDINGS

The monkey was euthanatized on da 43 following onset of clinical symptoms. By gross examination, all major organ systems, except for the brain, appeared normal. Congestion of the meningeal vessels was evident. A round, soft, necrotic, hemorrhagic area involving the left ventrolateral portion of the brain stem and meninges was noted. No exudative material was observed and all cultures were negative.

Tissue samples were fixed in neutral phosphate buffered 10% formalin and processed through paraffin. Sections were cut at 5-6 and stained with hematoxylin and eosin. Selected sections were also stained with tissue gram stain (Brown-Hopps) for bacteria and phosphotungstic acid hematoxylin (PTAH) for fibrin thrombi.

The major histologic lesions consisted of areas of necrosis and malacia in the brain stem and spinal cord in conjunction with vascular sclerosis and thrombosis. Multiple fibrin thrombi were also observed in kidney glomeruli. Tissue gram stains were negative for bacteria, while PTAH stains were positive for fibrin.

DISCUSSION

Seventy to ninety percent of bacterial meningitis in humans are caused by S. pneumoniae, Haemophilus influenzae, and Neissera meningitidis (4,5). Swartz and Dodge reported that pneumococci were the most common etiologic agents cultured when all age groups were considered (4). In nonhuman primates, S. pneumoniae, Pasteurella multocida, Klebsiella spp., and Mima polymorpha have been incriminated as the cause of meningitis (1-3,7-9). The incidence of pneumococcal meningitis in rhesus monkeys is unknown. Since necropsies were diagnostic in several reported cases where death occurred without previously observed clinical illness, it is probably more common than originally thought (2,3).

The association of pneumococcal pneumonia and meningitis is evident in monkeys and man (1-3,5). Cases of pneumococcal meningitis may occur because of hematogenous spread of the organism from pulmonary lesions. Keeling and McClure reported a case of pneumococcal meningitis in a chimpanzee which also had radiographic evidence of pneumonia (9). In this report, no radiographic or pathologic lesions of pneumonia were evident, and the source of meningeal infection was not determined. Since many of the reported cases of pneumococcal meningitis have occurred in newly imported monkeys, Fox and Rohovsky have stated that prophylactic treatment with antibiotics during conditioning may lower the morbidity and mortality of this disease in rhesus monkeys (2).

Clinically ill monkeys with pneumococcal meningitis are generally first noticed because of a neurological abnormality. A variety of neurological signs (head tilt, incoordination, nystagmus, pupillary constriction, blindness, gerneralized muscle tremors, abnormal consensual light reflexes, paralysis, and convulsions) have been observed in several cases of pneumococcal meningitis (1,2,9). In this case, definite neurological signs were not observed until 40 hr after initial examination. The signs initially observed were anorexia, depression, and a behavioral abnormality which was interpreted as abdominal pain. Abnormal behavior similar to that observed in our case has also been reported (1); however, the monkey also displayed a head tilt. The occurrence of similar behavioral changes as described in this report and by Fox and Soave should compel one to perform a CSF examination (1). Because of the monkey's behavior, the possibility of pancreatitis or some other abdominal disorder was investigated. Serum amylase levels were normal, but abdominal contrast radiographs revealed severe gastrointestinal hypomotility. The cause of the hypomotility, whether neurologically induced or due to the hypokalemic state of the animal, is not known. Although peritonitis has been reported to occur in conjunction with pneumococcal meningitis (3), there was no gross or histologic evidence of peritonitis in this case.

The treatment of pneumococcal meningitis is extensive, prolonged, and may be unrewarding. In addition to vigorous antibiotic therapy, IV fluids must be maintained at least until the monkey can resume adequate oral intake. Fox and Soave used a combination of penicillin and cephalordine to treat a rhesus monkey; however, after 12 da of therapy the monkey was euthanatized because of residual neurological signs (1).

Antibiotic therapy of another rhesus monkey which recovered consisted of IV chloromycetin, 70 mg/kg, 3 times daily (t.i.d.) for 3 da, followed by 100 mg/kg IM t.i.d. for 3 da (2). Keeling and McClure used a combination of penicillin (8,000,000 units/da) and ampicillin (4 g/da) to treat a chimpanzee for pneumococcal meningitis (9). Etterdorf treated pneumococcal meningitis in humans with sulphadiazine, chloramphenical, and penicillin (10). A multiple antibiotic regimen has been advocated when the etiological agent is unknown, or a resistant organism is present (11). Generally, in humans, it has been accepted that IV penicillin (4-24 million units/da) is the antibacterial agent of choice for pneumococcal meningitis, with IV chloramphenical (25-100 mg/kg/da) or erythromycin (25-4000 mg/kg/da) as alternative choices (5). Penicillin therapy for S. pneumoniae is usually continued for a minimum of 10-14 da (5,10). In the case presented, a combination of continuous IV penicillin (2,000,000 units/da) and chloromycetin (100 mg/kg/da) divided into 3 IV doses was used as described previously. Marked clinical improvement was observed after 48 hr of treatment. Most human cases will show definite improvement within 96 hr (5). Keeling and McClure similarly observed a marked improvement on the 3rd day of illness (9). CSF cultures performed at this time usually are negative (5,9).

In this animal, initial clinical signs consisted of depression, anorexia, and an abnormal behavorial pattern. No CNS signs were observed initially; however, nystagmus and generalized muscle tremors developed 2 days later. Cerebrospinal fluid examination was performed and a diagnosis of bacterial meningitis was made. Cultures of the CSF identified the etiologic agent as S. pneumoniae. Extensive antibiotic

treatment with penicillin and chloromycetin was initiated and marked improvement occurred. However, following the cessation of the antibiotic therapy a gradual loss of CNS function was noted which progressed until the animal had to be destroyed. Subsequent CSF examination and cultures during the course of this case and at necropsy did not demonstrate the presence of any bacterial organism. The major CNS histological lesion was necrosis and malacia in the brain stem and spinal cord in conjunction with vascular thrombosis of the meningeal and spinal cord vessels. Thrombosis of the kidney glomeruli was also observed.

The subsequent neurological problem observed in this monkey was most likely due to the S. pneumoniae infection. Although the active infection was apparently controlled, the residual lesions noted were probably due to the vascular changes and thrombosis of the meningeal and spinal cord vessels. Similar cases with vascular damage and subsequent thrombosis leading to residual CNS damage due to pneumococcal meningitis have been reported previously (1,2). The observation of glomerular thrombosis in this monkey suggests that disseminated intravascular coagulation may have occurred (12).

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TABLE 1-Laboratory data in a rhesus monkey with pneumococcal meningitis

Parameter	Value					
	Da 2	Da 3	Da 5	Da 16	Da 23	Da 43
RBC ($x10^6$)	4.9	4.0	5.0	4.9	4.9	5.5
Hb (gm %)	11.0	10.2	10.8	10.5	10.2	12.6
PCV (%)	37	30	35	34	33	37
WBC ($x10^3$)	12.5	24.9	35.5	8.7	9.3	6.5
Neutrophils (%)	71	75	78	78	64	77
Lymphocytes (%)	23	19	21	18	31	20
Eosinophils (%)	0	0	0	0	2	0
Basophils (%)	0	0	0	0	0	1
Monocytes (%)	6	2	1	4	3	2
Glucose (mg%)	111	101	67	140	100	108
BUN (mg%)	17			23	10	34
Sodium (mEq/Liter)	135	151	142	145	147	147
Potassium (mEq/Liter)	2.6	1.8	3.5	3.8	3.8	3.2
Chloride (mEq/Liter)	90	96				92
Total protein (gm%)	6.5			7.5		7.6
Albumin (gm%)	2.2			3.6		4.2
Globulin (gm%)	4.3			3.9		3.4
SGOT (B-MIU)	14					
SGPT (B-MIU)	10					
Amylase (Somogyi Units)	116	202				
Cerebrospinal fluid						
WBC (cells/mm^3)		2,500			14	13
Neutrophils (%)		83			2*	1*
Lymphocytes (%)		17			12*	23*
Total protein (mg%)		1,060				165
Glucose (mg%)		4				125
Staining		G+ cocci			Negative	Negative
Culture		S. pneumoniae			No growth	No growth

*Total number of each cell type on slide.